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# Fatty acid profile, *trans*-octadecenoic, α-linolenic and conjugated linoleic acid contents differing in certified organic and conventional probiotic fermented milks

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ABSTRACT

Development of dairy organic probiotic fermented products is of great interest as they associate ecological practices and benefits of probiotic bacteria. As organic management practices of cow milk production allow modification of the fatty acid composition of milk (as compared to conventional milk), we studied the influence of the type of milk on some characteristics of fermented milks, such as acidification kinetics, bacterial counts and fatty acid content. Conventional and organic probiotic fermented milks were produced using *Bifidobacterium animalis* subsp. *lactis* HN019 in co-culture with *Streptococcus thermophilus* TA040 and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB340. The use of organic milk led to a higher acidification rate and cultivability of *Lactobacillus bulgaricus*. Fatty acids profile of organic fermented milks showed higher amounts of *trans*-octadecenoic acid (C18:1, 1.6 times) and polyunsaturated fatty acids, including *cis*-9 *trans*-11, C18:2 conjugated linoleic (CLA-1.4 times), and α-linolenic acids (ALA-1.6 times), as compared to conventional fermented milks. These higher levels were the result of both initial percentage in the milk and increase during acidification, with no further modification during storage. Finally, use of bifidobacteria slightly increased CLA relative content in the conventional fermented milks, after 7 days of storage at 4 °C, whereas no difference was seen in organic fermented milks.



## 1. Introduction

Organic methods of food production have gained increased public interest over the past two decades, mainly in the western world. Organic and conventional dairy productions differ in feeding regimens, use of antibiotics, chemotherapeutic treatments, and handling of the animals (Collomb et al., 2008). Organic milk is produced in an agro-system under more constrained conditions in which the use of synthetic livestock additives or other artificial inputs, as well as genetically modified organisms, are forbidden. This production relies on ecological practices that prohibit the use of antibiotics, hormones and any synthetic chemical fertilizers (Toledo, Andrén, & Bjorck, 2002).

Milk is an excellent source of lactose, dairy proteins such as caseins and whey proteins and calcium and other minerals and trace elements. According to Ellis et al. (2006) there is little or no difference between organic and conventional milk samples when considering their carbohydrate, protein and mineral contents. Conversely, significantly higher amounts of polyunsaturated fatty acids (PUFA), conjugated linoleic (CLA) and *n*−3 fatty acids are found in organic milk (Collomb et al., 2008). This is also confirmed by Butler, Stergiadis, Seal, Eyre, and Leifert (2011), who indicated that fatty acid profile and antioxidant content of milk are influenced by management (organic or conventional), season and brands. The distribution of these fatty acids in milk is important as it confers different characteristics to the milk (Ekinci, Okur, Ertekin, & Guzel-Seydim, 2008).

Among the unsaturated fatty acids, the relative concentrations of three main long chain fatty acids (LCFA) differed according to the kind of milk. Conjugated linoleic acid, an isomer of linoleic acid (C18:2), has gained considerable attention due to its potentially beneficial biological effects (Gnädig, Xue, Berdeaux, Chardigny, & Sebedio, 2003), including anticarcinogenic, antiatherogenic, antidiabetic and immune stimulation. The *trans* fatty acids content in milk represents about 2% of total fatty acids, which can be increased to 4–10% of total fatty acids by enhancing dietary unsaturated oils content in the cow's diet. *Trans*-vaccenic acid, known as (*E*)-11-octadecenoic acid (C18:1 *trans*-11, or TVA), is the main *trans* fatty acid isomer found in the fat of ruminants and in dairy products, such as milk and yogurts (Santora, Palmquist, & Roehrig, 2000). It participates in CLA production, through enzymatic action of Δ-9-desaturase in mammary glands (Gnädig et al., 2003), and contributes to the supply of human body CLA (Butler et al., 2011). It is also an intermediate fatty acid of the CLA biohydrogenation pathway

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(Bergamo, Fedeli, Iannibelli, & Marzillo, 2003). Finally, α-linolenic acid (ALA), the major omega-3 fatty acid in milk, has been related to an ability to exert anti-arrhythmic effects in the heart, a positive impact on neurological function (by limiting central nervous system injury) and protection against coronary heart disease (Barceló-Coblijn & Murphy, 2009). It is also the dietary precursor for three long-chain omega-3 polyunsaturated fatty acids (LC-PUFA) synthesis: eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) (Brenna, Salem, Sinclair, & Cunnane, 2009).

Production of fermented milks, using bifidobacteria, is a big challenge in the dairy industry because milk, on the whole, is not a suitable matrix for the growth of lactic and probiotic bacteria since they lack essential proteolytic activity (Oliveira, Sodini, Remeuf, & Corrieu, 2001). Interest in bifidobacteria for human health is related to their survival through the intestinal tract and to their role in stimulating the immune system and prevention of microbial gastroenteritis (Foligne et al., 2007; Hols et al., 2005). In addition, CLA production by bifidobacteria was shown to be a possible mechanism for their health-enhancing properties (Oh et al., 2003).

Until now, few studies have explored the effect of organic milk on the growth of bifidobacteria and yogurt starters. To our knowledge, only the work of Florence et al. (2009) describes the acidification profile, fatty acids contents, and chemical composition of organic and conventional milks fermented by bifidobacteria in co-culture with *Streptococcus thermophilus*. These authors detected higher protein and iron concentrations in organic fermented milks, although no difference was observed in the initial milk. In addition, they found higher relative concentrations of TVA and CLA in organic fermented milks. From this information, it seems that a better knowledge about acidification kinetics and milk composition of organic and conventional fermented milk products is needed. In this context, this study aimed at characterising the behaviour of bifidobacteria and yogurt starters during organic and conventional milk fermentation. Their impacts on milk composition, in terms of overall fatty acid composition, and *trans*-octadecenoic, conjugated linoleic and α-linolenic acid relative contents, were determined and compared, during fermentation and cold storage of the fermented milks.

## 2. Material and methods

### 2.1. Milks

Commercial organic (Naturallis, São Paulo, Brazil) and conventional (Batavo, São Paulo, Brazil) UHT whole milks were purchased from a local supermarket. They were heat-treated at 85 °C for 15 min in a water-bath (Lauda, Type A100, DR. R. Wobser GmbH & Co. KG, Germany), under constant stirring. They were cooled down to 10 °C and stored overnight at 4 °C before manufacture of fermented milks.

Skimmed milk powder (Molico, Nestlé, São Paulo, Brazil) was reconstituted at 10% (w/w) and heat-treated at 121 °C for 15 min. It was used for inoculum preparation.

### 2.2. Preparation of cultures

Three commercial freeze-dried strains of probiotic and yogurt cultures were employed: *S. thermophilus* TA040 (Danisco, Dangé-Saint-Romain, France), *Lactobacillus delbrueckii* subsp. *bulgaricus* LB340 (Danisco, Madison, WI) and *B. animalis* subsp. *lactis* HN019 (Danisco, Madison, USA).

Each lyophilized strain was weighed and rehydrated in 50 ml of sterilized skimmed milk at 42 °C for 15 min before use, as recommended by the manufacturer. One mililiter of each rehydrated culture was inoculated into 500 ml of organic and conventional milk, allowing initial counts of 6.0 $\log_{10}$ colony forming units (CFU)/ml.

### 2.3. Experimental procedure

Organic and conventional UHT heat-treated milks were tempered at 42 °C, divided into two batches, and inoculated with two combinations of starter cultures. Yogurt was achieved by inoculating both *S. thermophilus* TA040 (50%) and *Lactobacillus bulgaricus* LB340 (50%) and probiotic fermented milk was prepared by inoculating these two strains (33% each) and *Bifidobacterium lactis* HN019 (33%). Inoculated milk samples were incubated at 42 °C in a thermostatically controlled water bath until pH reached 4.5. The pH and the acidification rate (dpH/dt, in upH/min) of each microbial blend were monitored by using the Cinac system (Ysebaert, Frépillon, France). The time to reach pH 4.5 ($t_{pH\ 4.5}$, in hours) was used to differentiate the mixed cultures. After reaching of pH 4.5, the fermentations were stopped by rapid cooling in an ice bath to 10 °C. The samples were dispensed into 50 ml polypropylene cups, thermally sealed using Selopar equipment (BrasHolanda, Pinhais, Brazil) and stored at 4 °C until required for analysis. The samples were prepared in duplicate, and the experiment was replicated twice on different days.

Before fermentation, at final fermentation time and after 7 days of storage at 4 °C, the cultivability (CFU/ml) of yogurt and probiotic bacteria, the fatty acids profile of milk and fermented milks, including *trans*-octadecenoic acid, CLA and ALA relative contents, were determined.

### 2.4. Chemical composition of milks

Fat, proteins, total solids content and density were determined with an ultrasonic Ekomilk milk analyzer (Eon Trading, Stara Zagora Bulgaria). Titratable acidity was analyzed as recommended by AOAC (1995) and lactose concentration was determined according to the Lane and Eynon method, based on the reduction of copper (AOAC, 1995). A digital potentiometer (Mod.8603, Mettler-Toledo, Scherzenbach, Switzerland) was used for pH measurements. All analyses were duplicated.

### 2.5. Cultivability measurements

The CFU counts ($\log_{10}$ CFU/ml) were determined in triplicate. *S. thermophilus* and *L. bulgaricus* were respectively plated onto M17 lactose agar and MRS agar (Oxoid, Basingstoke, UK), previously acidified to pH 5.4 with acetic acid. *B. lactis* was enumerated in RCA (Oxoid, Basingstoke, UK) treated with 2 μg/ml of dicloxacillin (pH 7.1) and 0.3 g/l of aniline blue (InLab, São Paulo, Brazil). They were incubated at 37 °C for 48 h under anaerobic conditions (AnaeroGen, Oxoid, Basingstoke, UK). CFU were counted after anaerobic incubation at 37 °C for 72 h of at least four replicates.

### 2.6. Fatty acids extraction and analysis

The lipids were extracted from organic and conventional UHT milks, yogurts and probiotic fermented milks, according to the ISO method 14156 (ISO, 2001), which is a dedicated method for extraction or separation of lipids and liposoluble compounds from milk and milk products. Fatty acid methyl esters (FAME) of milk lipids were prepared by transesterification according to the ISO method 15884 (ISO, 2002), that consists of a base-catalyzed methanolysis of the glycerides, followed by a neutralization with crystalline sodium hydrogen sulfate to avoid saponification of esters.

Analyses of FAME were carried out in a gas chromatograph, model 3400CX (Varian, Walnut Creek, CA, USA) equipped with a

split-injection port, a flame-ionization detector and a software package for system control and data acquisition (model Star Chromatography Workstation version 5.5). Injections were performed in a 30 m long fused silica capillary column with 0.25 mm internal diameter, coated with 0.25 μm Chrompack CP-Wax 52CB (Chrom-Tech, Apple Valley MN, USA). Helium was used as carrier gas at a flow rate of 1.5 ml $min^{-1}$ and a split ratio of 1:50. The injector temperature was set at 250 °C and the detector at 280 °C. The oven temperature was initially set at 75 °C for 3 min, then programmed to increase to 150 °C at a rate of 37.5 °C $min^{-1}$, and then to 215 °C at a rate of 3 °C $min^{-1}$ (Luna et al., 2004). Samples (1 μl) were injected manually after a dwell-time of *ca* 2 s. Qualitative fatty acid composition of the samples was determined by comparing the retention times of the peaks with those of standards 05632 and 189-19 (Sigma, Chemical Co., St. Louis, MO, USA). The relative content of each FAME was calculated from the area of each peak, and expressed as a percentage, according to the official method, Ce 1–62 (AOCS, 1997). Results were grouped and expressed as percentages of short chain fatty acids (SCFA – C4:0 and C6:0), medium chain fatty acids (MCFA – C8:0 to C15:0), long chain fatty acids (LCFA – C16:0 to C18:3), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), according to Ackman (2007). All samples were analyzed in quadruplicate.

### 2.7. Statistical analysis

General Linear Models (GLM), multifactor analyses of variance (ANOVA) and multiple comparison tests were done, using Statistica 8.0 (Statsoft, Tulsa, USA) in order to determine statistical significance of differences among samples. Mean values were compared using the Newman Keuls test at $P < 0.05$.

## 3. Results and discussion

### 3.1. Compositions of organic and conventional milks

The chemical compositions, expressed as percentage (%), were similar for conventional and organic milks. The contents of fat (3.0 ± 0.05%), total solids (11.7 ± 0.09%) and lactic acid (0.15 ± 0.01%) were similar in both milks, as measured before fermentation (day 0). Conversely, protein (2.4 ± 0.0%) and lactose (4.7 ± 0.1%) concentrations were significantly lower in organic milk than in conventional milk (2.8 ± 0.1% and 4.9 ± 0.1%, respectively). The chemical compositions of organic and conventional cow milks, found in the present study, were comparable to those reported by (Sola-Larrañaga & Navarro-Blasco, 2009). By contrast, Toledo et al. (2002) reported similar levels of lactose but higher fat and protein concentrations. Differences in milk composition can be attributed to management system, season, and sampling periods in which the milk was purchased (Butler et al., 2011).

Table 1 summarizes the percentage of total identified fatty acid composition of the four kinds of fermented milks, before (0) and after fermentation, and after 1 day and 7 days of storage at 4 °C. The fatty acid composition of conventional and organic milks differed according to the kind of milk used for the fermentation. Their distribution according to chain length allowed separation of short chain (SCFA), medium chain (MCFA) and long chain fatty acids (LCFA). The saturation degree allowed classification of the fatty acids into saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

The main fatty acids encountered in milk corresponded first to saturated fatty acids, such as myristic acid (C14:0, 12.1–12.7%), palmitic acid (C16:0, 28.9–31.9%) and stearic acid (C18:0, 9.6–12.2%). Second, monounsaturated fatty acids were also found. Among them, oleic acid (C18:1 *cis*-9, 21.3–21.8%), palmitoleic acid (C16:1 *cis*-9, 1.5–1.9%) and *trans*-octadecenoic acid (*trans*-C18:1, 2.1–3.3%) were the more abundant. Third, polyunsaturated fatty acids were detected. The PUFA fraction was mostly composed of linoleic acid (*cis*-9 *cis*-12 C18:2, 1.6–1.9%), conjugated linolenic acid (*cis*-9 *trans*-11, CLA, 0.7–1.0%) and α-linolenic acid (*cis*-9 *cis*-12 *cis*-15 C18:3, ALA, 0.3–0.5%). PUFA and MUFA concentrations were, in this study, lower (2.5–3.5% and 27–28%, respectively) than those found by Rodríguez-Alcalá, Harte, and Fontecha (2009) in cow milk (5.7% for PUFA and 32.9% for MUFA). As a consequence, higher relative contents of SFA were found in the present study, 68–71% as compared to 60% obtained by Rodríguez-Alcalá et al. (2009).

From Table 1, the fatty acid composition results, expressed as% of total fatty acids, differed, according to chain length, in organic and conventional milks, as measured before fermentation. The relative content of short chain fatty acids (SCFA; C4:0 and C6:0) was lower in organic milk (5.6% instead of 6.4%) than in conventional milk. The medium chain fatty acid (MCFA; C8:0–C15:0) percentage was slightly lower in organic milk (difference of 0.6%). These data are in agreement with those reported by Collomb et al. (2008) who also did not find significant difference according to the long chain length (LCFA; C16:–C18:3) in organic and conventional milks.

The proportion of saturated fatty acids (SFA) was slightly higher in conventional milk (+2%). Conversely, for Collomb et al. (2008) and Ellis et al. (2006), organic and conventional milks did not significantly differ with respect to SFA. MUFA proportion was always lower in conventional fermented milks (−2%). Nevertheless, these results conflict with those obtained by Ellis et al. (2006), who found higher amounts of MUFA in conventional milks. More specifically, *trans*-C18:1 relative content was 1.6 times higher in organic products (Fig. 1A), in agreement with data reported by (Bergamo et al., 2003). After all, the percentage of PUFA fraction was 1.3 times higher in organic products, than in conventional milks, as previously reported by Ellis et al. (2006). Among these PUFA, the linoleic acid (LA – C18:2) was higher in organic milk, with 1.9 ± 0.02% instead of 1.6 ± 0.01% for conventional products. The initial relative contents of CLA (1.0 ± 0.01%) and ALA (0.5 ± 0.00%) were 1.4- and 1.6-times higher in organic milk (Fig. 1B and C). Even if Ellis et al. (2006) did not confirm that as a general rule, similar findings were reported by Bergamo et al. (2003) and Collomb et al. (2008).

Finally, the main difference observed in fatty acid composition of conventional and organic milks was related to the higher unsaturated fatty acid content in organic milk. This could be ascribed to the feeding regimen of the cows, as demonstrated by Bergamo et al. (2003), Butler et al. (2011) and Collomb et al. (2008).

### 3.2. Fermentation profile

The acidification profiles of yogurt made with *S. thermophilus* TA040 and *L. delbrueckii* subsp. *bulgaricus* LB340, and probiotic fermented milk containing the same yogurt culture plus *B. animalis* subsp. *lactis* HN019, in organic and conventional UHT milks, are shown on Fig. 2.

A similar acidification profile was observed for yogurt culture in both milks (Fig. 2A). Even if the initial pH differed slightly (pH 6.54 ± 0.01 conventional milk, instead of pH 6.65 ± 0.01 in organic milk), the higher rate of acidification in organic milk ($15.3 \times 10^{-3}$ upH/min) than in conventional milk ($11.7 \times 10^{-3}$ upH/min) (Fig. 2B) allowed the final pH to be reached at the same time ($t_{pH4.5}$ = 6.2 ± 0.3 h in both fermented milks). From Fig. 2B, two maximum acidification rates were observed whatever the kind of milk. This was explained by Pernoud, Fremaux, Sepulchre, Corrieu, and Monnet (2004), who demonstrated that *S. thermophilus* is a urease-positive species, thus allowing urea conversion into ammonia and carbon dioxide. This transitory ammonia synthesis neutralized lactic acid, thus explaining the temporary pH stabilization,

**Table 1**
Evolution of identified fatty acid methyl esters composition (%) in organic and conventional milks fermented by yogurt cultures or probiotic yogurt cultures.

| Kind of milk | Sample | Time (days) | SCFA | MCFA | LCFA | SFA | MUFA | PUFA |
|---|---|---|---|---|---|---|---|---|
| Organic | Y | 0 | $5.51 \pm 0.18^{abc}$ | $21.7 \pm 0.36^{a}$ | $71.8 \pm 0.25^{abc}$ | $68.9 \pm 0.20^{ab}$ | $27.9 \pm 0.26^{ab}$ | $3.36 \pm 0.04^{bc}$ |
| | Y | 1 | $5.08 \pm 0.16^{a}$ | $22.1 \pm 0.19^{ab}$ | $72.8 \pm 0.312^{bc}$ | $68.3 \pm 0.18^{a}$ | $28.1 \pm 0.18^{b}$ | $3.61 \pm 0.02^{cd}$ |
| | Y | 7 | $5.29 \pm 0.14^{ab}$ | $21.9 \pm 0.16^{ab}$ | $72.8 \pm 0.30^{bc}$ | $68.1 \pm 0.24^{a}$ | $28.2 \pm 0.21^{b}$ | $3.65 \pm 0.04^{d}$ |
| | PY | 0 | $5.61 \pm 0.13^{abc}$ | $22.7 \pm 0.15^{ab}$ | $71.7 \pm 0.25^{abc}$ | $68.9 \pm 0.15^{ab}$ | $27.7 \pm 0.13^{ab}$ | $3.39 \pm 0.01^{bc}$ |
| | PY | 1 | $5.10 \pm 0.08^{a}$ | $21.7 \pm 0.33^{a}$ | $73.2 \pm 0.36^{c}$ | $68.4 \pm 0.32^{a}$ | $28.0 \pm 0.19^{ab}$ | $3.60 \pm 0.05^{bcd}$ |
| | PY | 7 | $5.48 \pm 0.22^{abc}$ | $22.1 \pm 0.26^{a}$ | $72.5 \pm 0.46^{abc}$ | $68.5 \pm 0.13^{a}$ | $27.9 \pm 0.13^{ab}$ | $3.60 \pm 0.02^{bcd}$ |
| Conventional | Y | 0 | $6.31 \pm 0.38^{bc}$ | $24.0 \pm 0.84^{b}$ | $70.0 \pm 0.97^{ab}$ | $71.1 \pm 0.87^{c}$ | $26.6 \pm 0.40^{a}$ | $2.62 \pm 0.10^{a}$ |
| | Y | 1 | $5.00 \pm 0.09^{a}$ | $21.6 \pm 0.16^{a}$ | $73.4 \pm 0.21^{c}$ | $69.0 \pm 0.18^{ab}$ | $28.2 \pm 0.16^{b}$ | $2.58 \pm 0.08^{a}$ |
| | Y | 7 | $5.56 \pm 0.25^{abc}$ | $23.47 \pm 0.61^{ab}$ | $71.0 \pm 0.85^{abc}$ | $70.4 \pm 0.37^{bc}$ | $27.0 \pm 0.32^{ab}$ | $2.61 \pm 0.04^{a}$ |
| | PY | 0 | $6.30 \pm 0.41^{bc}$ | $24.0 \pm 0.89^{b}$ | $70.1 \pm 1.01^{ab}$ | $71.1 \pm 0.85^{c}$ | $26.7 \pm 0.49^{a}$ | $2.58 \pm 0.08^{a}$ |
| | PY | 1 | $5.44 \pm 0.21^{abc}$ | $22.8 \pm 0.60^{ab}$ | $71.4 \pm 0.93^{abc}$ | $69.7 \pm 0.50^{abc}$ | $27.3 \pm 0.49^{ab}$ | $2.69 \pm 0.14^{a}$ |
| | PY | 7 | $4.89 \pm 0.01^{a}$ | $21.7 \pm 0.04^{a}$ | $73.4 \pm 0.04^{c}$ | $69.2 \pm 0.01^{ab}$ | $28.1 \pm 0.03^{b}$ | $2.71 \pm 0.02^{a}$ |

*Abbreviations:* Y = yogurt culture; PY = probiotic yogurt culture; Short Chain fatty acid (SCFA, C4:0 to C6:0); Medium Chain fatty acid (MCFA, C8:0 to C15:0); long chain fatty acid (LCFA, C16:0 to C18:3); SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; 0 = days 0 (before fermentation), 1 (1 day after fermentation) and 7 = (7 days storage at 4 °C). Mean values ($N = 4$), ± standard deviation with different letters in the same column are significantly different ($P \leqslant 0.05$).

which resulted in these two peaks. This phenomenon has a direct impact on acidification profiles, due to natural variation of the urea level in milk (Hols et al., 2005). The previous phenomenon, engendered by urease activity, was not observed in the acidification profile of organic milk fermented with probiotic plus yogurt culture (Fig. 2C) that displayed a typical sigmoid behavior. This could be explained by the lower urea level in organic milk than in conventional milk, as previously reported by Toledo et al. (2002).

By considering the mixed culture, including *B. lactis* HN019, the use of organic milk increased acidification rates as compared to conventional milk (Fig. 2B and D). This difference allowed the acidification of organic milk to be significantly more rapid ($18.6 \times 10^{-3}$ upH/min instead of $14.2 \times 10^{-3}$ upH/min, $P < 0.05$) with bifidobacteria, lactobacilli and streptococci than with only yogurt bacteria. The time to reach pH 4.5 was 6.2 ± 0.2 h in organic milk instead of 6.9 ± 0.1 h in conventional milk, which was significantly different ($P < 0.05$). This result is in agreement with those of Florence et al. (2009) who reported shorter fermentation time using binary cultures of *B. animalis* subsp. *lactis* and *S. thermophilus* in organic milks. It may be supposed that the strain *B. lactis* HN019 required specific nutriments that were found in organic milk, but not in conventional milk.

### 3.3. Bacterial concentrations during fermented milks production and storage

Bacterial growth differed according to both type of milk and mixed culture composition. Indeed, microbial interactions can result, either in stimulation, delay, inhibition, or the absence of effects, depending on bacterial species and strains (Roy, 2005; Vinderola, Costa, Regenhardt, & Reinheimer, 2002).

Growth of *S. thermophilus* TA040 occurred during the first two hours of fermentation, resulting from its rapid lactose assimilation, in agreement with earlier works of Béal and Corrieu (1994). Final concentrations of *S. thermophilus* achieved at the end of the fermentation, ranged from 8.9 to 9.1 $\log_{10}$ CFU/ml, with no significant differences ($P > 0.05$) between the two different kinds of milk and types of cultures employed.

Growth of *L. bulgaricus* LB340 started after four hours of fermentation, in agreement with previous studies (Oliveira et al., 2009). Final concentrations were significantly higher ($P < 0.05$) in organic milk fermented by yogurt culture (8.1 ± 0.03 $\log_{10}$ CFU/ml) as compared to the other conditions (7.8 ± 0.03 $\log_{10}$ CFU/ml). A positive effect of organic milk was thus demonstrated on *L. bulgaricus* growth, which can be related to the higher poly-unsaturated fatty acid content (1.3-times higher) in this kind of milk than in conventional milks. Higher viability of lactic acid bacteria was previously achieved when the ratio between unsaturated and saturated fatty acids was increased (Béal, Fonseca, & Corrieu, 2001; Castro, Teixeira, & Kirby, 1995). This could be explained by the stereochemistry of the double bounds of unsaturated fatty acids, which control membrane fluidity during exposure to the adverse environmental conditions found in fermented milks, such as low pH or low temperature. Moreover, the more rapid acidification observed in organic milk could be another factor of *L. bulgaricus* improvement.

No significant difference ($P > 0.05$) was noted for *B. lactis* HN019 growth in organic and conventional milk. Bacterial counts at the end of fermentation were equal to 7.9 ± 0.03 $\log_{10}$ CFU/ml and 8.1 ± 0.06 $\log_{10}$ CFU/ml for organic and conventional milk, respectively.

Final concentrations of *L. bulgaricus* and *S. thermophilus*, at the end of the cultures, were not significantly influenced by the presence of the probiotic culture *B. lactis* HN019 ($P > 0.05$). This result differs from those obtained by Vinderola et al. (2002) on the one hand and Donkor, Henriksson, Vasiljevic, and Shah (2006) on the other, who demonstrated that *L. bulgaricus* and *S. thermophilus* were either inhibited or stimulated by *Bifidobacterium* strains, respectively. This contradictory information indicates that the interactions between yogurt bacteria and *Bifidobacterium* are strongly strain-dependent.

Growth of *B. lactis* HN019 in milk remained weak, as final concentrations were around 8.1 ± 0.06 $\log_{10}$ CFU/ml. This result agreed with those reported by Vinderola et al. (2002), who showed that addition of probiotic cultures to yogurt starters generally results in slower growth of the probiotic strains than if they were added alone to milk. This was explained, first by the accumulation of lactic and acetic acids that affect the viability of bifidobacteria and, second by the low proteolytic activity of these bacteria (Roy, 2005).

Finally our results demonstrated that fermentation was mainly ascribable to *S. thermophilus*, which reached a final concentration 1 log higher than *L. bulgaricus* and *B. lactis*. Only a slight effect of the type of milk was noticed on the growth of *L. bulgaricus,* when associated with *S. thermophilus*, organic milk leading to a better growth of this species. The faster growth of starter cultures allowed rapid acidification, which resulted in reduced availability of nutrients; thus, probiotic cultures do not have time to grow extensively (Roy, 2005).

By considering the bacterial concentrations measured after 7 days of storage at 4 °C, evidently the kind of milk did not affect the survival of the three bacterial species that were stable during cold storage. Concentrations were equal to 8.8 ± 0.2 $\log_{10}$ CFU/ml for *S. thermophilus* TA040, 7.6 ± 0.2 $\log_{10}$ CFU/ml for *L. bulgaricus* LB340 and 7.9 ± 0.1 $\log_{10}$ CFU/ml for *B. lactis* HN019, in both milks. Moreover, no significant difference ($P > 0.05$) was observed with the counts measured just after fermentation. This result differs from that obtained by Donkor et al. (2006), who indicated that

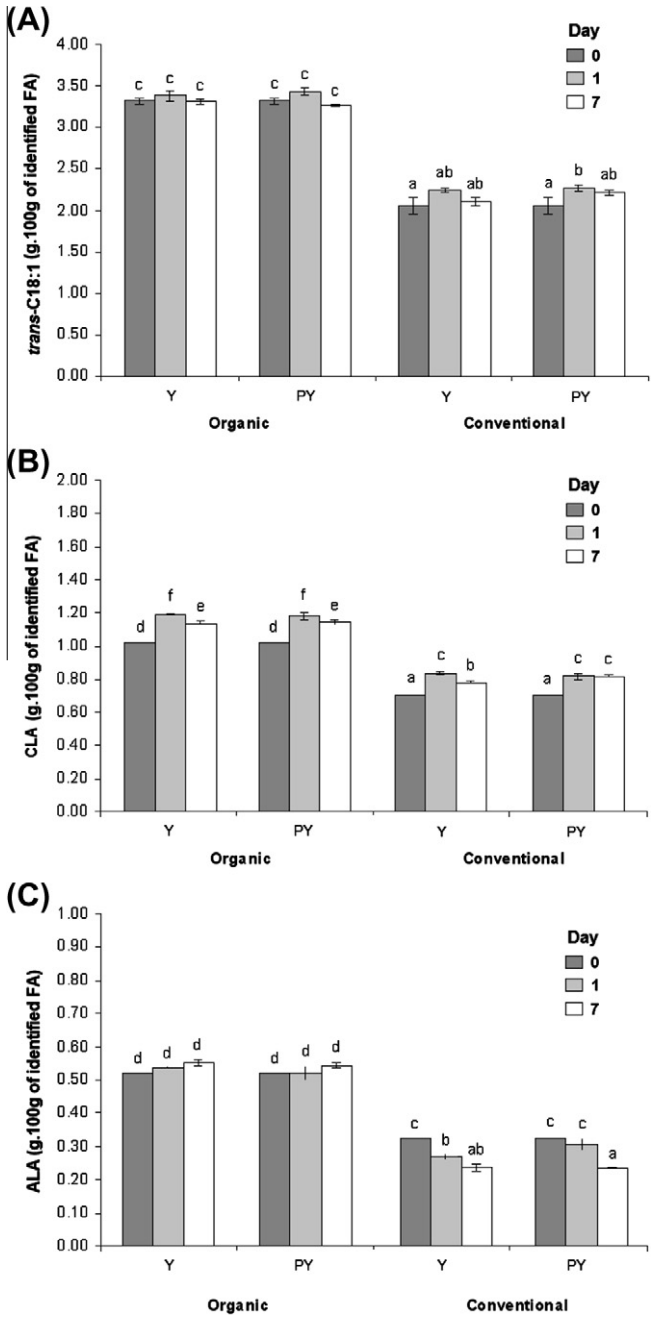


**Fig. 1.** Evolution of *trans*-octadecenoic acid (*trans*-C18:1, A), conjugated linoleic acid (CLA, B) and α-linolenic acid (ALA, C) relative contents in organic and conventional milks during fermentation by *Streptococcus thermophilus* TA040 and *Lactobacillus bulgaricus* LB340 (Y), and *Streptococcus thermophilus* TA040, *Lactobacillus bulgaricus* LB340 and *Bifidobacterium animalis* subsp. *lactis* HN019 (PY). 0: day 0 (before fermentation); 1: day 1 (1 day after fermentation); 7: day 7 (7 days storage at 4 °C); Means ($n = 4$) with different letters are significantly different; $P \leqslant 0.05$.

the viability of *L. bulgaricus* Lb1466 was enhanced in the presence of probiotic organisms during cold storage. It was thus strain-dependent.

### 3.4. Fatty acid profiles of milk during fermentation and storage

The fatty acid profiles varied during milk fermentation, as a result of the kind of milk and the type of starter culture. In contrast, no modification was observed during storage at 4 °C for 7 days.

The relative content of SCFA was slightly reduced during fermentation ($P < 0.05$), in both conventional and organic fermented products, independently of co-culture employed. During cold storage for 7 days, the SCFA of the fermented milks did not change anymore, whatever the type of milk. These data differ from those reported by Ekinci et al. (2008), who observed higher amounts of short chain fatty acids in products fermented with other bacterial species. In conventional milks, independently of the co-culture used, the MCFA concentration decreased during fermentation, whereas no significant difference was observed during 7 days of storage at 4 °C. In organic milk, the MCFA relative contents did not change during fermentation and after 7 days of cold storage. In addition, no significant difference ($P \geqslant 0.05$) was pointed out between organic and conventional milks. Nevertheless, relative concentrations of C14:1 and C15:0 were slightly higher ($P < 0.05$) in fermented conventional milks, which agrees with the study of Butler et al. (2011) who found higher concentration of MCFA in conventional milk. Finally, a significant increase in LCFA concentration was observed during fermentation (between 1 and 2%), but not during storage at 4 °C, for both organic and conventional fermented milks. The relative contents of LCFA did not show significant difference ($P > 0.05$) between the two kinds of milks, in agreement with recent findings (Collomb et al., 2008; Ellis et al., 2006). Among these LCFA, higher relative contents of C16:0; C16:1 and C17:0 were found in conventional products, whereas relative amounts of C18:0 and C18:2 were higher in organic fermented milks.

In addition to these results, that concerned the chain length of milk fatty acids, important changes were observed in the fatty acid saturation degree during fermentation ($P < 0.05$). In conventional milk, the proportion of saturated fatty acids (SFA) strongly decreased during fermentation (1–2%), whereas it diminished only slightly in organic milk (~0.4%). As a result of SFA level decrease during fermentation, the relative concentration of MUFA increased in conventional milk (1%) but not in organic milk (Table 1).

The levels of MUFA, measured after fermentation, were practically alike for both milks in our study. The percentage of PUFA increased during fermentation in organic milk (~0.2%) but remained stable in conventional milk. These results are in agreement with those obtained by Florence et al. (2009) with the cultures of *S. thermophilus* and four strains of *B. lactis*. They could be explained either by the different balance with MUFA or SFA, or by the synthesis of some polyunsaturated fatty acids by the bacteria (Oh et al., 2003).

The relative percentages of SFA, MUFA and PUFA at day 7 remained close to those measured at day 1 (Table 1). At 4 °C, the metabolic activity of the bacteria was reduced as a consequence of the low temperature, and no more change occurred in the fatty acid content as a result of their metabolic activity. This result is in agreement with those reported by Rodríguez-Alcalá & Fontecha, 2007 with CLA-fortified dairy products. They showed that the relative contents of SFA, MUFA and PUFA remained stable during storage. In contrast, Van de Guchte et al. (2006) observed that the total $n-3$ PUFA concentration decreased slightly during storage of conventional fermented milks. This difference can be ascribed to the different strains used.

Moreover, no significant effect of the type of starter culture was noticed on the chain length of milk fatty acids. The relative proportions of each group of fatty acids varied in the same way, whether or not the probiotic culture was added to the yogurt culture. The same conclusion was achieved by comparing the fatty acid composition after 7 days of storage at 4 °C, which was not affected by the starter and remained stable. Finally, fermentation allowed increasing MUFA relative concentration in conventional milk, whereas organic fermented milks were characterized by an increase of PUFA relative contents. This indicates that the fatty acid composition of the fermented milk was the result of initial saturation degree, as well as modification during fermentation. This result confirmed

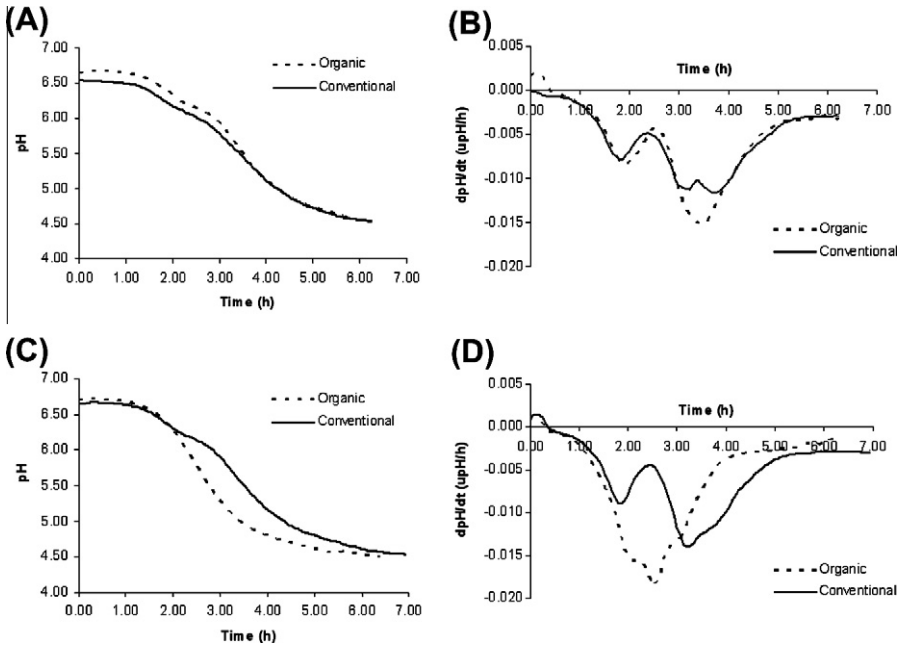


**Fig. 2.** Acidification kinetics in organic and conventional milks incubated at 42 °C until pH 4.5, with (A and C) *Streptococcus thermophilus* TA040 and *Lactobacillus bulgaricus* LB340, and (B and D) *Streptococcus thermophilus* TA040, *Lactobacillus bulgaricus* LB340 and *Bifidobacterium animalis* subsp. *lactis* HN019. - - - - Organic milk; —— conventional milk.

those obtained by Van de Guchte et al. (2006) with conventional fermented milks enriched, or not, with PUFA or whey proteins.

From these results, differences were observed according to fatty acid chain length and saturation degree by comparing organic and conventional fermented milks. We ascribe these differences to both initial milk composition and modification by fermentation. The initial fatty acid profile of milk was primarily determined by the balance of fatty acids in the feeding regimen and the extent of rumen hydrogenation and mammary desaturase activity that differed in the two systems of dairy production (Butler et al., 2011). Moreover, fatty acid composition of fermented milks was affected by growth and corresponding enzymatic activities of bacterial cells, which differed according to the milk, as a result of initial fatty acid profile (Ekinci et al., 2008; Kim & Liu, 2002).

In contrast, no differences were noted during cold storage of fermented milks. This fact may be due to the slower metabolic activity of bacteria at low temperature (Béal et al., 2001).

### 3.5. Evolution of trans-octadecenoic acid (trans-C18:1), conjugated linoleic acid (CLA) and α-linoleic acid (ALA) relative contents during fermentation and cold storage

During fermentation, *trans*-C18:1 relative concentration (Fig. 1A) showed a 20% increase in conventional fermented milks, with no significant difference among the starter cultures, whereas an enhancement of 8% was observed in organic milk. As the initial relative concentration of *trans*-C18:1 was 1.6 times higher in organic milk, the final *trans*-C18:1 percentage in the fermented milks was the result of both initial milk composition and modification during fermentation. It is interesting to maintain a high relative content of *trans*-C18:1 as it participates in CLA production in the human (Butler et al., 2011; Gnädig et al., 2003) and acts as an intermediate fatty acid in the biohydrogenation pathway (Bergamo et al., 2003). During storage of the fermented products, the *trans*-C18:1 concentration remained stable, whatever the kind of milk and starters used. Finally, after 7 days storage at 4 °C, it was higher in organic fermented milks (3.3 ± 0.03%) than in conventional milks (2.2 ± 0.03%).

During fermentation, CLA relative content significantly increased ($P < 0.05$), at different levels in organic (17%) and conventional (12%) milks (Fig. 1B). This was explained by Ekinci et al. (2008), who indicated that enzymatic reactions occurred in the biohydrogenation pathway, thus increasing CLA level during the production of fermented products. Similar results were reported by Oliveira et al. (2009) in fermented milks, whereas no change was observed in probiotic fermented products made with conventional milk, as reported by Van de Guchte et al. (2006). As these authors used different strains, this behavior was thus strain-dependent. The difference between conventional and organic fermented milks found in our study was considered as significant ($P < 0.05$). The CLA relative concentration was higher in organic fermented milks (1.2 ± 0.01%) than in conventional fermented milks (0.8 ± 0.01%) (Fig. 1B), in accord with previous results (Oliveira et al., 2009). This higher CLA relative content in organic fermented products was the result of both initial CLA percentage in milk and changes during fermentation. In addition to these results, CLA relative concentration did not significantly vary in fermented milks according to the co-cultures. This result indicates that *B. lactis* HN019 had no effect on CLA relative content, and that the variations observed during fermentation could be ascribed to *S. thermophilus* or *L. bulgaricus*, as suggested by Lin (2003). Finally, the CLA percentage slightly decreased during cold storage of three of the fermented milks ($P < 0.05$), that may be related to the activation of reduction steps in the biohydrogenation pathway (Kim & Liu, 2002). However, by considering the conventional fermented milk with yogurt starters and bifidobacteria, a significant increase of relative CLA content was observed.

Fig. 1C shows that, during fermentation, ALA level did not vary significantly in organic milk (0.5 ± 0.02%), for the two kinds of culture. In contrast, a significant decrease ($P < 0.05$) was noted during fermentation and storage of conventional milk products (from 0.38 ± 0.02% to 0.30 ± 0.02%). These results are not in agreement with those of Van de Guchte et al. (2006), who showed that the content of ALA was not affected during storage of conventional fermented milks at 4 °C, which can be attributed to the different strains used. No significant difference was noticed between the

two kinds of starters at the end of the fermentation. Finally, the ALA content in the fermented milks mainly resulted from its initial concentration in milk and from variation during fermentation and storage. During 7 days of storage at 4 °C, strong difference was observed between the two kinds of fermented milks. The ALA content remained high and stable in organic milk (0.54 ± 0.02%), whereas it decreased from 0.30 ± 0.02% to 0.24 ± 0.01% in conventional milk. This decrease can be correlated with the increased levels of C18:0 and C18:1, independently of the co-culture used, as a result of modification of biohydrogenation and desaturation pathways (Destaillats, Trottier, Galvez, & Angers, 2005).

## 4. Conclusions

Our study has demonstrated that the use of organic milk allowed more rapid acidification and provided higher PUFA content in the fermented milks and this was related to an improvement of *L. bulgaricus* growth. In contrast, the growth of *S. thermophilus* and *B. lactis* HN019 was not affected by the type of milk. Bacterial concentrations remained stable after 7 days of storage at 4 °C.

Acidification process also provided *trans*-C18:1 and CLA enhancement, together with ALA decrease, at different levels in conventional and organic milks. This result indicates that bacterial metabolism modified the relative fatty acid milk composition. By combining these differences with the initial fatty acid composition of organic and conventional milks, which depended on variations in dairy diet manipulation, evidently organic fermented milks had higher relative amounts of *trans*-C18:1 (×1.6), CLA (×1.4) and ALA (×1.6), than had conventional fermented milks at the end of fermentation and after storage at 4 °C. Consequently, the fatty acid content of the fermented milks was the result of two factors: initial milk composition and modification during fermentation as a result of bacterial metabolic activities. The higher relative amounts of *trans*-C18:1, CLA and ALA in organic fermented milks and lower levels of SFA may be considered as desirable from a nutritional perspective.

In the future, it will be necessary to identify the specific role of each bacterial species, in pure cultures, in order to understand the biochemical mechanisms that support the changes in fatty acid composition in the fermented milks.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.07.026.

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